

ORIGINAL ARTICLE

Asthma and Lower Airway Disease

Contribution of airway eosinophils in airway wall remodeling in asthma: Role of *MMP-10* and *MET*

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Funding information

The U-BIOPRED project is supported through an Innovative Medicines Initiative Joint Undertaking under grant agreement 115010, resources of which are composed of financial contributions from the European Union's Seventh Framework Programme

Abstract

Background: Eosinophils play an important role in the pathophysiology of asthma being implicated in airway epithelial damage and airway wall remodeling. We determined the genes associated with airway remodeling and eosinophilic inflammation in patients with asthma.

Methods: We analyzed the transcriptomic data from bronchial biopsies of 81 patients with moderate-to-severe asthma of the U-BIOPRED cohort. Expression profiling was performed using Affymetrix arrays on total RNA. Transcription binding site analysis used the PRIMA algorithm. Localization of proteins was by immunohistochemistry.

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(FP7/2007-2013) and European Federation of Pharmaceutical Industries and Associations companies' in-kind contributions (www.imi.europa.eu).

Results: Using stringent false discovery rate analysis, MMP-10 and MET were significantly overexpressed in biopsies with high mucosal eosinophils (HE) compared to low mucosal eosinophil (LE) numbers. Immunohistochemical analysis confirmed increased expression of MMP-10 and MET in bronchial epithelial cells and in subepithelial inflammatory and resident cells in asthmatic biopsies. Using less-stringent conditions (raw *P*-value < 0.05, log₂ fold change > 0.5), we defined a 73-gene set characteristic of the HE compared to the LE group. Thirty-three of 73 genes drove the pathway annotation that included extracellular matrix (ECM) organization, mast cell activation, CC-chemokine receptor binding, circulating immunoglobulin complex, serine protease inhibitors, and microtubule bundle formation pathways. Genes including MET and MMP10 involved in ECM organization correlated positively with submucosal thickness. Transcription factor binding site analysis identified two transcription factors, ETS-1 and SOX family proteins, that showed positive correlation with MMP10 and MET expression.

Conclusion: Pathways of airway remodeling and cellular inflammation are associated with submucosal eosinophilia. MET and MMP-10 likely play an important role in these processes.

KEYWORDS

asthma, eosinophil, mast cell, MET, MMP10

1 | INTRODUCTION

Asthma is a complex disease that is heterogeneous in its clinical presentation. In addition, its inflammatory component may vary between asthmatic subjects with the recognition of eosinophilic inflammation as being a specific feature of a particular type of asthma associated with a late-onset nonatopic asthma, presence of nasal polyps, and chronic airflow obstruction and likely to be treated with oral corticosteroid therapy.¹ Asthmatic patients with eosinophilia as evidenced by an increase in blood or sputum eosinophil count are also at an increased risk of exacerbations and poorly controlled asthma,² and are also more likely to respond to corticosteroid therapy³ and to specific anti-eosinophilic therapies such as anti-IL-5 immunomodulators.⁴

These observations indicate that eosinophils may play an important role in the pathophysiology of asthma. Eosinophils have been implicated in damaging the airway epithelium through the release of reactive oxygen species, eosinophil cationic protein, and eosinophil peroxidase and have also been implicated in airway wall remodeling changes such as the increase in reticular subbasement membrane by direct interaction of eosinophils with fibroblasts.^{5,6} In addition, IL-5 activation of eosinophils may lead to the production of cationic proteins that activate TGFβ, MMP, and PDGF release from bronchial epithelial cells.⁷ Indeed, anti-IL-5 antibody therapy in patients with asthma can lead to a reduction in extracellular matrix proteins in the subbasement membrane.⁸

We hypothesized that eosinophils contribute to airway wall remodeling and inflammation in asthma. To this end, we analyzed the transcriptome of bronchial biopsies of patients with asthma in

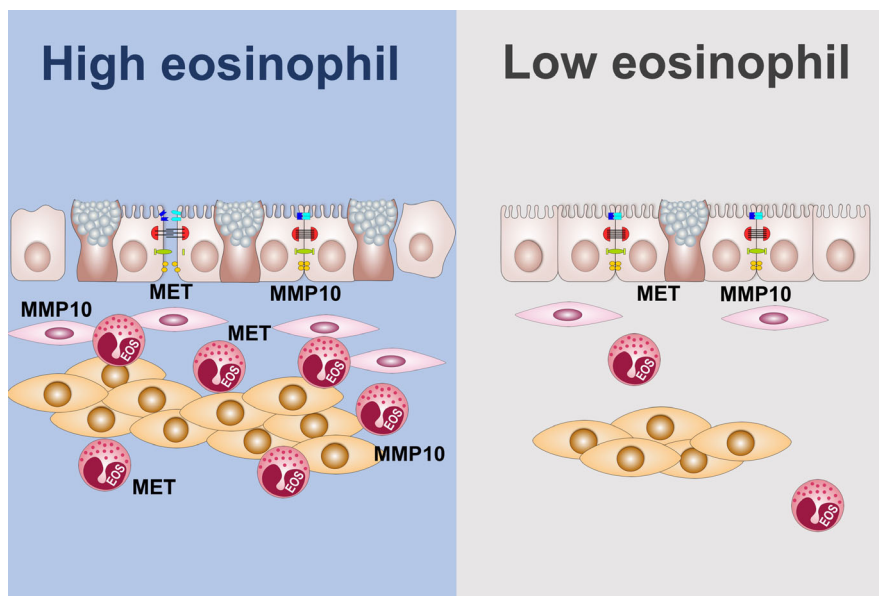
relation to the degree of eosinophilic inflammation using data collected in the European U-BIOPRED cohort.⁹ We determined the differentially expressed genes (DEGs) in the bronchial mucosa from patients with high levels of eosinophils compared to low levels of eosinophil density in their bronchial mucosa. We confirmed dysregulation of MMP10 and MET protein expression using immunohistochemistry and identified the transcription factors that control the expression of their genes.

2 | MATERIALS AND METHODS

2.1 | Clinical data of patients with bronchial biopsies suitable for immunohistochemistry

In this study, of 158 participants who underwent bronchoscopic procedure, 137 and 136 biopsies were suitable for immunohistochemistry and transcriptomic microarray analysis, respectively; where 123 were qualified for both, of whom 81 participants were moderate-to-severe asthma and 41 healthy controls (Table S1) from 11 European centers of the U-BIOPRED study.⁹ Expression profiling of the bronchial biopsies was performed using Affymetrix U133 Plus 2.0 (Affymetrix, Santa Clara, CA, USA) microarrays.

Prebronchodilator spirometry, exhaled nitric oxide (FeNO), sputum differential cell count, allergic status assessed by either skin prick tests or RAST test to specific aeroallergens, serum total IgE, and differential blood count were measured.⁹ The study was approved by the Ethics Committees of each recruiting center, and all participants gave written informed consent.



GRAPHICAL ABSTRACT

Analysis of differentially expressed genes in eosinophil-high biopsies of asthma yielded MMP10 and MET as potential drivers of airway wall remodeling. Important pathways include extracellular matrix organization, mast cell activation, C-C receptor binding, and regulation of leukocyte activation. Bronchial eosinophils are important drivers of airway wall remodeling in asthma. MMP10: Matrix metalloproteinase 10 gene. MET: Hepatocyte growth factor receptor gene.

2.2 | Immunohistochemistry of U-BIOPRED bronchial biopsies

Samples of bronchial biopsies from paraformaldehyde-preserved sections (6 μ m) were stained with monoclonal antibodies against CD3, CD4, CD8, neutrophil elastase, and EG2. Cell counts were performed in a blinded fashion and expressed as number of positive cells/mm². These results have already been reported.¹⁰

2.3 | Immunohistochemical analysis of MET and MMP-10 in bronchial biopsies

In order to confirm the presence of MET and MMP10 in the airways of patients with asthma, bronchial biopsies kept at the National Heart and Lung Institute Biobank were obtained from six normal and 10 asthma (five mild and five severe) subjects. Paraffin sections were immunostained with primary rabbit anti-MET antibody (1:200 dilution) (Abcam, Cambridge, UK, ab51067) and rabbit anti-MMP10 (1:100 dilution) (Abcam, ab189210) antibodies, and MET- and MMP10-positive cells were detected using peroxidase staining method. Nonspecific rabbit IgG polyclonal-isotype control (Abcam, ab171870) was used as a control primary antibody.

The immunostaining intensity for MET and MMP10 on bronchial epithelium was semi-quantitatively scored from 0 to 3 (0—negative, 1—weak, 2—moderate, and 3—strong staining). All available epithelial and subepithelial areas from 2 to 3 biopsies of each subject were assessed and scored or counted. Depending on the size of the biopsies, more than 15 fields using $\times 40$ objective lens were scored for

epithelial staining intensity for each subject. More than 1.5-mm² subepithelial area was measured and counted for MET+ and MMP10+ inflammatory cells for each subject. Cell counts were expressed as the number of cut cell profiles with a visible nucleus per mm² of the subepithelium.

2.4 | Microarray mRNA analysis

Expression profiling was performed using Affymetrix U133 Plus 2.0 microarray (Affymetrix, Santa Clara, CA, USA) on total RNA extracted from bronchial biopsies. RNA purity (RIN > 9.5) was measured by Agilent Bioanalyser (Agilent, Santa Clara, CA, USA). Raw data were quality-assessed and preprocessed by robust multi-array average normalization using Almac Pipeline and Pre-processing Toolbox (Almac, Craigavon, UK), and batch/technical effects were adjusted as covariates using general linear model.

2.5 | Gene set variation analysis (GSVA) and transcription factor binding site analysis

To facilitate biological interpretation, functionally related genes were grouped as a gene set and their degree of expression summarized by GSVA (Bioconductor R package GSVA) as a quantitative enrichment score for each patient as previously described.¹¹ In order to analyze for transcription factor (TF) binding sites, PRIMA (PRomoter Integration in Microarray Analysis) algorithm was used as previously described.¹² Briefly, the algorithm used a Position weight matrix-based model to scan the promoter region of the target genes and

identified the hits of putative binding sites of each TF. In turn, the hits of each TF from the target genes were determined for their over-representation with respect to a genome-wide background setting.

2.6 | Statistical analysis

The datasets for this analysis were uploaded from the transSMART system, an open-source knowledge management platform for sharing research data¹³ supported by the European Translational Information and Knowledge Management Services (eTRIKS) project. All categorical variables were analyzed using Fisher's exact test. Student's *t* test was used for continuous variables with normal distribution, otherwise Wilcoxon rank-sum test was used. A linear model for microarray data (Bioconductor R package limma) with false discovery rate (FDR) correction was used for differential expression analysis. All *P*-values < 0.05 were considered statistically significant.

3 | RESULTS

3.1 | Transcriptomic profile in asthmatic bronchial biopsies

We first determined differences in the transcriptome of bronchial biopsies between patients with asthma (*n* = 81) and normal controls (*n* = 41). We extracted a set of 251 DEGs and used unbiased principal component analysis (PCA) using these DEGs from the asthmatic cohort to identify significant clinical profiles associated with the variance of the gene expression. Upon visual inspection, no single clinical profile could account for the major variation of the first and second PC in the asthmatic cohort including the severity of asthma (Figure S1). A similar result was seen with hierarchical clustering where two major clusters were identified with no significant

enrichment of specific clinical profiles including asthma severity was noted (Figure S2A, chi-squared test, *P* = 0.740). However, there was a trend toward a significant difference in the submucosal eosinophil profile between the two clusters (Figure S2B, Wilcoxon sign-rank test, *P* = 0.091). Using GSVA, we also found that there was little difference in expression levels of activation gene signatures for neutrophils, macrophages, and CD4 + T cells between the mucosal eosinophil-high and eosinophil-low patients (data not shown).

3.2 | Transcriptomic biomarker of eosinophilic inflammation

Because the comparison of asthmatic to normal controls did not yield any satisfactory clinical profiles, we next focused on the transcriptomic differences in bronchial biopsies between eosinophil-high and eosinophil-low patients. To this end, the K-mean clustering method was applied, which yielded four homogeneous mucosal eosinophil clusters (low, medium-low, medium-high, and high; Figure S3A), as determined by the within-group sum of squares, using the eosinophil counts in bronchial biopsies that have been previously reported in this cohort¹⁰ (Figure S3B). The low and medium-low clusters were further aggregated as the low mucosal eosinophil (LE) group (*n* = 48), and the medium-high and high clusters as the high mucosal eosinophil (HE) group (*n* = 33). The HE cohort had higher eosinophil counts compared to the LE cohort (9.8 vs 1.1 counts/mm², *P* = 2.2×10^{-9}). To define the transcriptomic biomarkers of asthmatic eosinophilic inflammation in bronchial biopsies, we analyzed the DEGs between the HE and LE groups. Upon multiple testing correction with FDR, two probe sets at 205680_at (log₂FC:1.31, adjusted *P*-value = 0.035; MMP10) and 211599_x_at (log₂FC:0.42, adjusted *P*-value = 0.035; MET) were found to be significantly expressed in the HE group (Figure 1A). There was a significant correlation between submucosal eosinophil counts and the expression of

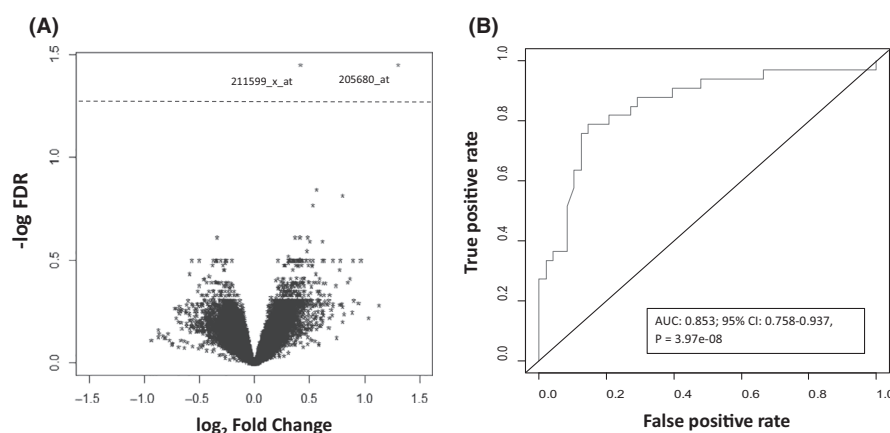


FIGURE 1 Biomarker analysis for mucosal eosinophilic inflammation. A, Volcano plot showing probe set/transcript-level expression comparing the HE against LE group, where x-axis is log₂ fold change, and y-axis is -log *P*-value adjusted for FDR. At FDR < 0.05 (red dashed line), two probe sets as 205680_at (log₂FC:1.31, adjusted *P*-value = 0.035, MMP10) and 211599_x_at (log₂FC:0.42, adjusted *P*-value = 0.035, MET) were differentially expressed. B, Receiver operating characteristic (ROC) curve showing good performance of MMP10 and MET probe sets as classifiers of mucosal eosinophilic inflammation using the support vector machine learning algorithm

MMP10 ($n = 65$; $r = 0.549$; $P = 2.23 \times 10^{-6}$) and of MET ($n = 65$; $r = 0.469$; $P = 8.28 \times 10^{-5}$). Applying these two probe sets to differentiate high bronchial eosinophil inflammation using a support vector machine learning algorithm, a good classification performance was achieved as shown by ROC curve analysis (AUC: 0.853; 95% CI: 0.758–0.937, $P = 3.97 \times 10^{-8}$; Figure 1B).

3.3 | Activation of extracellular matrix gene pathway in HE group

In order to understand the biological pathway(s) underlying all potential genes characteristic of the HE group, we defined the DEGs from the HE group using a nominal level of significance at $P < 0.05$ and an absolute value of $\log_2$ fold change > 0.5 . These genes were then subjected to an over-representation test for pathway analysis using three publicly available databases, GO, KEGG, and Reactome. Overall, 73 genes were found to be characteristic of the HE group (Figure 2A), which corresponded to several top-ranked pathways including extracellular matrix (ECM) organization (GO:0030198; $P = 2.2 \times 10^{-5}$, REAC:1474244; $P = 1.1 \times 10^{-4}$) and regulation of leukocyte activation (GO:0002696; $P = 0.0023$, Table S2). Thirty-three out of the 73 genes were enriched in all the pathways identified, whereas 40 were left unannotated. Six functionally relevant annotations were defined from the 33 genes, in which 10 genes including MMP10 were annotated as ECM organization, four as mast cell activation, six as C–C chemokine receptor binding, five as

circulating immunoglobulin complex, four as serine proteinase inhibitors (SERPINs), and five as microtubule bundle formation (Figure 2A and Table S3). The expression of this gene signature was not different between those on oral corticosteroid treatment compared to those who were not.

We next used gene set variation analysis (GSVA) to summarize each of the six upregulated functional annotated pathways into a signature score (ie, enrichment score) for each subject, thus enabling a quantitative analysis of mutual signature relationships across all participants with asthma. Using clustering analysis (Figure 2B), we showed that ECM, mast cell, and C–C chemokine were the three parallel signatures, as they presented strong inter-signature relationships with high correlations noted between the ECM and mast cell signature (Pearson's $r = 0.77$, $P = 4.4 \times 10^{-17}$; Figure 2C) and between the ECM and C–C chemokine signature (Pearson's $r = 0.68$, $P = 2.9 \times 10^{-12}$; Figure 2D).

3.4 | Implication of eosinophilic inflammation on patients with severe and mild/moderate asthma

We next determined the molecular implications of a high degree of eosinophil infiltration in bronchial biopsies with the severity level. The molecular findings are in line with the pathological and clinical profiles of the HE group, which showed significantly higher submucosal thickness of bronchial biopsies (9.6 vs 8.2 μm , $P = 0.006$), as well as peripheral blood eosinophilia (0.26 vs $0.15 \times 10^3/\mu\text{L}$,

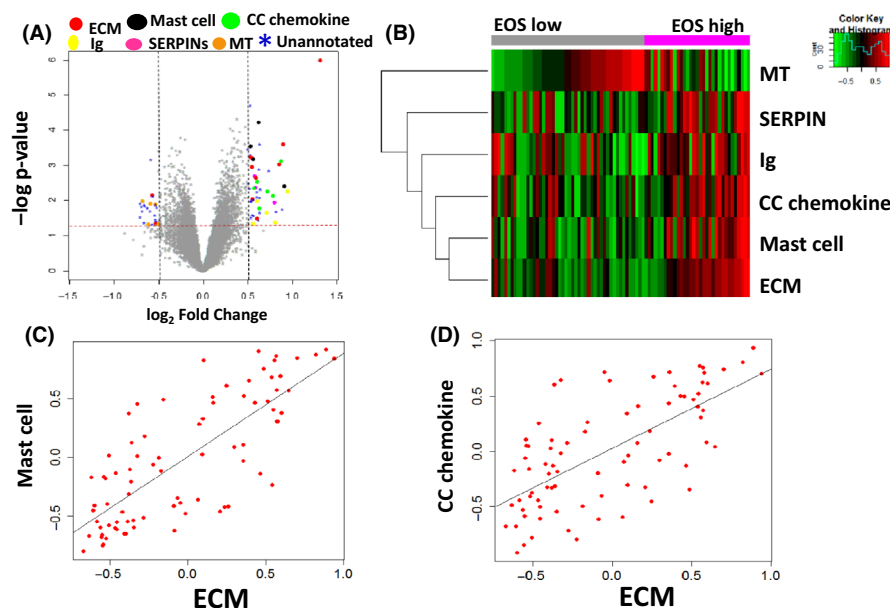


FIGURE 2 Functional/biological annotation of the genes characteristic of mucosal eosinophilic inflammation. A, Volcano plot showing gene-level expression comparing the HE vs LE group (black dashed line: absolute value of $\log_2$ fold change > 0.5 , red dashed line: nominal $P < 0.05$). Seventy-three genes were identified characteristic of the HE group, of which 10 were annotated as extracellular matrix (ECM, red) organization, four as mast cell activation (black), six as C–C chemokine receptor binding (green), five as circulating immunoglobulin complex (Ig, yellow), four as serine proteinase inhibitors (SERPINs, magenta), and five as microtubule bundle formation (MT, orange). B, Heat map showed clustering of six GSVA signatures at row side and column side corresponding to subjects of LE group (gray bar) and HE group (magenta bar). C, High correlation of GSVA signatures was identified between ECM and mast cell gene sets (Pearson's $r = 0.77$, $P = 4.4 \times 10^{-17}$) and D, between ECM and C–C chemokine gene sets (Pearson's $r = 0.68$, $P = 2.9 \times 10^{-12}$)

TABLE 1 Comparison of clinical features and submucosal cell profiles of the HE vs LE group in patients with severe asthma

Variables	HE (n = 20)	LE (n = 30)	Missing data, n (%)	P-value
Age (y)	52.2 ± 12.7	51.4 ± 11.4	-	0.814
Female	11 (55.0)	14 (46.7)	-	0.772
BMI	28.6 ± 5.2	31.2 ± 6.5	-	0.115
Smoking	4 (20.0)	10 (33.3)	-	0.479
Nasal polyp	6 (30.0)	12 (40.0)	-	0.673
Allergic rhinitis	12 (60.0)	13 (43.3)	-	0.386
Eczema	9 (45.0)	15 (50.0)	-	0.954
Oral corticosteroid use	10 (50.0)	12 (40.0)	-	0.684
Atopy	11 (55.0)	25 (83.3)	-	0.062
Exacerbations (per year)	3.0 (2.0-4.0)	2.5 (1.8-3.0)	14 (28.0)	0.603
FEV ₁ (% predicted)	64.9 (50.8-74.1)	70.5 (57.5-87.6)	-	0.076
Total serum IgE (IU/mL)	179.0 (68.1-458.5)	85.9 (31.7-351.0)	1 (2.0)	0.218
Blood eosinophil (10 ³ /μL)	0.30 (0.20-0.40)	0.19 (0.10-0.29)	-	0.026
Sputum eosinophil (%)	6.1 (0.7-23.9)	2.1 (0.2-14.3)	25 (50.0)	0.443
FeNO (ppb)	30.0 (18.3-49.0)	26.0 (17.0-47.0)	5 (10.0)	0.794
Serum periostin (ng/mL)	45.2 (41.9-52.8)	44.0 (38.9-53.7)	7 (14.0)	0.605
Serum CRP (mg/L)	1.5 (1.0-4.2)	5.0 (1.1-7.0)	1 (2.0)	0.094
Submucosal cell counts				
Eosinophil (mm ⁻³)	7.8 (5.7-13.4)	1.0 (0-2.2)	-	2.3 × 10 ⁻⁹
Neutrophil (mm ⁻³)	16.0 (6.3-22.2)	12.1 (8.4-17.1)	-	0.294
Macrophage (mm ⁻³)	4.1 (2.2-8.6)	2.0 (0.7-3.3)	-	0.005
CD4 T cell (mm ⁻³)	12.2 (7.8-15.4)	6.5 (4.3-11.8)	-	0.070
CD8 T cell (mm ⁻³)	20.7 (13.5-34.7)	12.7 (4.6-22.5)	-	0.099
Mast cell (mm ⁻³)	20.8 (15.8-34.1)	13.6 (4.6-23.3)	-	0.111
ASM fraction (%)	0.33 (0.26-0.39)	0.29 (0.22-0.42)	-	0.642
Thickness (μm)	10.0 (8.7-10.7)	8.0 (7.0-9.8)	9 (18.0)	0.019

ASM, airway smooth muscle; BMI, body mass index; CRP, C-reactive protein; EOS, eosinophil; FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; IgE, immunoglobulin E.

Data are presented as N (%) and mean (SD) or median (IQR).

$P = 0.005$) as compared to the LE group. In contrast, there was no difference in the proportion of patients with severe asthma between the HE and LE groups (60.6% vs 62.5%, $P = 1.000$, Table S4).

We therefore stratified these patients with asthma by severity and analyzed whether there were differential effects of eosinophilic inflammation in severe vs nonsevere asthma. In severe asthmatic subjects, HE showed significantly higher submucosal thickness (10.0 vs 8.0 μm, $P = 0.019$), peripheral blood eosinophilia (0.30 vs 0.19 × 10³/μL, $P = 0.026$), a trend toward lower FEV₁ predicted (64.9% vs 70.5%, $P = 0.076$) and frequency of atopy (55.0% vs 83.3%, $P = 0.062$, Table 1) as compared to the LE group. In mild/moderate asthmatic patients, the significantly higher submucosal thickness of bronchial biopsies was not seen in the HE group (9.1 vs 8.3 μm, $P = 0.302$), whereas the trend toward peripheral blood eosinophilia (0.24 vs 0.10 × 10³/μL, $P = 0.072$, Table 2) remained. In addition, the FeNO level was significantly higher in the HE (34.0 vs 19.0 ppb, $P = 0.029$, Table 2) than in the LE group, a finding which was not seen in severe asthma.

3.5 | Pathway analysis of HE group for patients with severe and mild/moderate asthma

We further analyzed the DEGs between HE and LE groups by analyzing the underlying biological pathways distinguishing the HE and LE groups in patients with severe and mild/moderate asthma independently. In patients with severe asthma, a total of 66 genes were identified, including MMP10, CCL26, and POSTN, which were all upregulated, and pathway enrichment analysis showed the top-ranked pathways as ECM organization (REAC:1474244; $P = 0.003$) axonemal dynein complex assembly (GO:0070286; $P = 0.007$) and microtubule bundle formation (GO:0001578; $P = 0.015$, Table S5). In contrast, in patients with mild/moderate asthma, a total of 259 genes were identified, including upregulation of SOX7, IL-33, TSLP, and MMP10, and the biological pathways also revealed the enrichment in ECM organization (REAC:1474244; $P = 9.2 \times 10^{-8}$), collagen biosynthesis (REAC: 1650814; $P = 1.3 \times 10^{-5}$), cell migration (GO: 0016477; $P = 2.5 \times 10^{-5}$) and interleukin-7 signaling (REAC: 449147; $P = 9.0 \times 10^{-5}$, Table S5).

TABLE 2 Comparison of clinical features and submucosal cell profiles of the HE vs LE group in patients with mild/moderate asthma

Variables	HE (n = 13)	LE (n = 18)	Missing, data, n (%)	P-value
Age (y)	38.9 ± 10.7	41.2 ± 15.4	-	0.617
Female	4 (30.8)	12 (66.7)	-	0.107
BMI	26.4 ± 4.7	27.7 ± 6.3	-	0.412
Smoking	0	0	-	-
Nasal polyp	0	2 (11.1)	-	0.616
Allergic rhinitis	7 (53.8)	8 (44.4)	-	0.879
Eczema	3 (23.1)	5 (27.8)	-	1.000
Atopy	10 (76.9)	14 (77.8)	-	1.000
Exacerbations (per year)	0	1.0 (1.0-1.0)	25 (80.6)	0.905
FEV ₁ (% predicted)	95.2 (73.5-104.0)	92.8 (72.2-102.0)	-	0.708
Total serum IgE (IU/mL)	125.0 (23.0-196.0)	58.8 (44.3-121.8)	-	0.378
Blood eosinophil (10 ³ /μL)	0.24 (0.20-0.30)	0.10 (0.10-0.25)	-	0.072
Sputum eosinophil (%)	0.45 (0.14-1.21)	0 (0-0.57)	18 (58.1)	0.498
FeNO (ppb)	34.0 (22.0-57.0)	19.0 (12.9-21.8)	-	0.029
Serum periostin (ng/mL)	44.0 (37.3-51.3)	43.5 (39.4-47.8)	4 (12.9)	0.683
Serum CRP (mg/L)	1.0 (1.0-2.3)	2.0 (1.0-5.0)	3 (9.7)	0.253
Submucosal cell counts				
Eosinophil (mm ⁻³)	6.1 (5.2-11.1)	0.7 (0-1.4)	-	2.7 × 10 ⁻⁶
Neutrophil (mm ⁻³)	11.1 (10.6-18.9)	9.1 (5.1-13.0)	-	0.230
Macrophage (mm ⁻³)	4.0 (2.2-7.3)	1.6 (0.7-3.5)	-	0.043
CD4 T cell (mm ⁻³)	14.8 (9.0-35.2)	9.1 (1.1-11.6)	-	0.012
CD8 T cell (mm ⁻³)	22.0 (13.6-35.4)	11.9 (5.0-19.3)	-	0.082
Mast cell (mm ⁻³)	29.0 (21.1-37.6)	19.6 (16.2-36.0)	-	0.246
ASM fraction (%)	0.30 (0.25-0.32)	0.35 (0.25-0.47)	-	0.367
Thickness (μm)	9.1 (8.0-9.7)	8.3 (7.0-9.3)	4 (12.9)	0.302

ASM, airway smooth muscle; BMI, body mass index; CRP, C-reactive protein; EOS, eosinophil; FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; IgE, immunoglobulin E.

Data are presented as N (%) and mean (SD) or median (IQR).

3.6 | Transcription factor binding site analysis for MMP10 and MET

Because the ECM signature, with MMP10 as its most highly expressed gene, drove the biomarker and functional pathway analysis, we explored the regulation of these genes within the ECM signature by analyzing associated transcription factors. To this end, we applied a Position weight matrix-based model to scan the promoter region (2000 bp upstream and 200 bp downstream of transcription start site) of the eight upregulated ECM genes. A set of 26 putative transcription factors (TFs) were identified whose binding sites were significantly enriched in the eight gene promoters. We specifically focused on the 16 TFs (Table S6) that had binding sites at the MMP10 promoter and interrogated whether their gene expression was correlated with the expression of MMP10 as well as other ECM genes. Among the 16 TFs analyzed, we confirmed the correlation of the TF ETS1 gene with MMP10 (Pearson's $r = 0.44$, $P = 4.0 \times 10^{-5}$; Figure S4A) and with CTSG (Pearson's $r = 0.32$, $P = 0.0036$; Figure S4B) as well as a negative relationship of NKX3A gene with MMP10 (Pearson's $r = -0.32$, $P = 0.0036$; Figure S4C). Three TFs (SOX, GATA6, and HNF4) were

enriched at the MET promoter site, but only the SOX family of TFs was significantly correlated with MET expression (Figure S4D).

3.7 | Immunohistochemical localization of MET and MMP-10

In order to examine the potential role of MMP10 and MET in airway wall remodeling in asthma, we performed immunohistochemical analysis of these proteins in bronchial biopsies. Bronchial cells including basal epithelial cells, subepithelial polymorph and mononuclear inflammatory cells, fibroblasts, and endothelial cells were stained positively and diffusely for both MET and MMP10. MET and MMP10 were expressed constitutively in the bronchial mucosa of normal controls (Figure 3A and B) and were more highly expressed in patients with asthma (Figure 3C and D), respectively. The scores of epithelial MET and MMP10 positivity were higher in asthmatic than that in healthy subjects ($P = 0.042$ and 0.0017 , Figure 4A and B, respectively). Counts of subepithelial MET- and MMP10-positive inflammatory cells trended toward significance and were significantly greater in asthma compared to normal ($P = 0.073$ and 0.042 , Figure 4C and D, respectively).

4 | DISCUSSION

We found 73 differentially expressed genes that were characteristic of patients with high bronchial tissue eosinophilic asthma compared to the low bronchial tissue eosinophilic group. Pathway analysis was driven by 33 of these 73 genes and defined ECM organization, mast cell activation, CC-chemokine receptor binding, circulating immunoglobulin complex, serine protease inhibitors, and microtubule bundle formation as key pathways. These pathways were associated with eosinophilic airway inflammation, increased blood and tissue macrophages, low systemic C-reactive protein levels, and increased basement membrane thickness. *MMP10* and *MET* were the only genes which differentiated low from high eosinophilic inflammation in biopsies when more stringent criteria were applied, and both *MET* and *MMP10* proteins were more highly expressed in airway epithelial cells and some submucosal inflammatory cells in asthma.

In our study, we used pathway analysis to highlight previously identified aspects of asthmatic inflammation in bronchial biopsies. Mast cells are seen more commonly in airway smooth bundles of patients with asthma compared to healthy nonasthmatic participants¹⁴; furthermore, eosinophils can produce the profibrogenic cytokines, TGF β and b-FGF-2, and also serine proteases.¹⁵ Mast cells may have an effect on airway wall remodeling, smooth muscle hypertrophy, and mucus hypersecretion.¹⁶ It is also clear that CC-chemokines produced by epithelial cells and mast cells can not only recruit and activate infiltrating immune cells but also contribute to airway wall remodeling by inducing the proliferation of airway smooth muscle cells and activate the release of profibrogenic cytokines on human lung fibroblasts.^{17,18} Our results also support a role for submucosal eosinophils in terms of extracellular matrix organization and mast cell activation, suggesting that there may be bi-directional interactions between eosinophils and mast cells. Mast cells are found in close proximity to eosinophils, and physical interactions between those cells have been reported.^{19,20}

Both *MMP10* and *MET* have been implicated in lung cancer pathogenesis. *MMP10* represents a critical lung cancer stem cell gene,²¹ while *MET* is a proto-oncogene.²² The product of *MET* is hepatocyte growth factor receptor (HGFR), and its ligand, hepatocyte growth factor (HGF), that upon binding to its receptor leads to receptor dimerization and phosphorylation of its tyrosine kinase domain resulting in activation of important pathways that have been implicated in asthma such as PI3K-Akt signaling, Ras-MAP kinase cascade, STAT 3, and NF- κ B complex. These cascades can promote a variety of cellular function including proliferation and protection from apoptosis.^{23,24} These signal transduction pathways have an important role in wound repair and hepatic regeneration, with an important role in liver development,²⁵ raising a potential role in airway wall remodeling. HGF suppresses Th1 and Th2 immune responses²⁶ and induces secretion of MIP-1 β , MIP-2 α , IL-6, IL-8, and IL-10 in monocytes.^{27,28} The emerging role of *MET* in mediating epithelial-mesenchymal transition has received increasing attention with studies revealing the plasticity of epithelial cells with transformation into myofibroblasts that in turn produces excessive amount of extracellular matrix and collagen that contributes to airway fibrosis.^{29,30} This transition may involve *MET*-HGF signaling cascades.^{31,32}

In an integrative genomic analysis of airflow obstruction, pathway enrichment analysis led to the identification of several biological modules including extracellular matrix processes, with *MMP-10* as a candidate gene.³³ The protective role of *MMP-10* in the development of cigarette smoke-induced emphysema in a knock-out mouse model has also been reported,³³ but its role in asthma remains to be determined. *MMP10* is upregulated in small airways in COPD.³⁴ *MMP10* gene expression is upregulated in airway epithelial cells infected with rhinovirus³⁵ and respiratory syncytial virus.³⁶ *MMP10* may drive the conversion of pro-inflammatory M1-biased macrophages toward immunosuppressive M2-biased cells, and, in the context of airway remodeling, *MMP-10* also controls the activation of ECM-degrading activity in M2 macrophages.³⁷

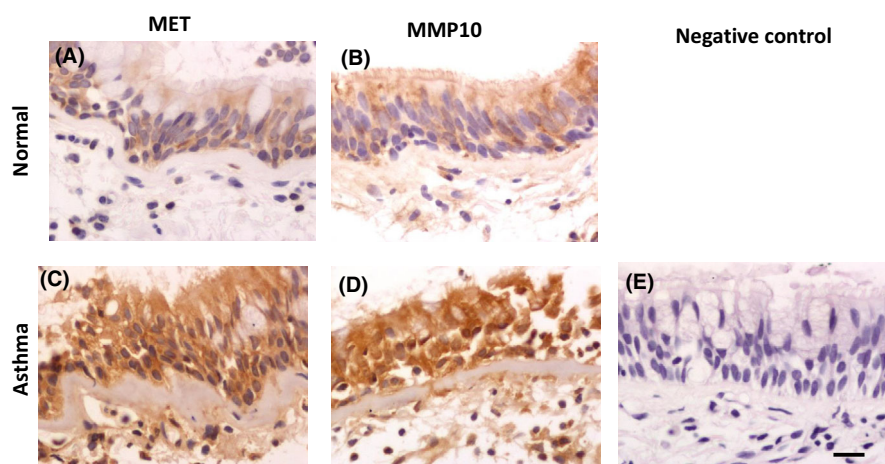


FIGURE 3 Immunohistochemistry for *MET* and *MMP-10* in bronchial biopsy sections. There is weaker immunostaining and fewer subepithelial positive cells in a normal subject compared to an asthmatic subject for both *MET* (A and C for normal and asthmatic, respectively) and *MMP-10* (B and D for normal and asthmatic, respectively). E, shows negative control with absence of signal. Internal scale bar = 20 μ m for all panels. Figures are representative of staining of samples from 6 healthy nonasthmatic (normal) subjects and 10 asthmatic subjects

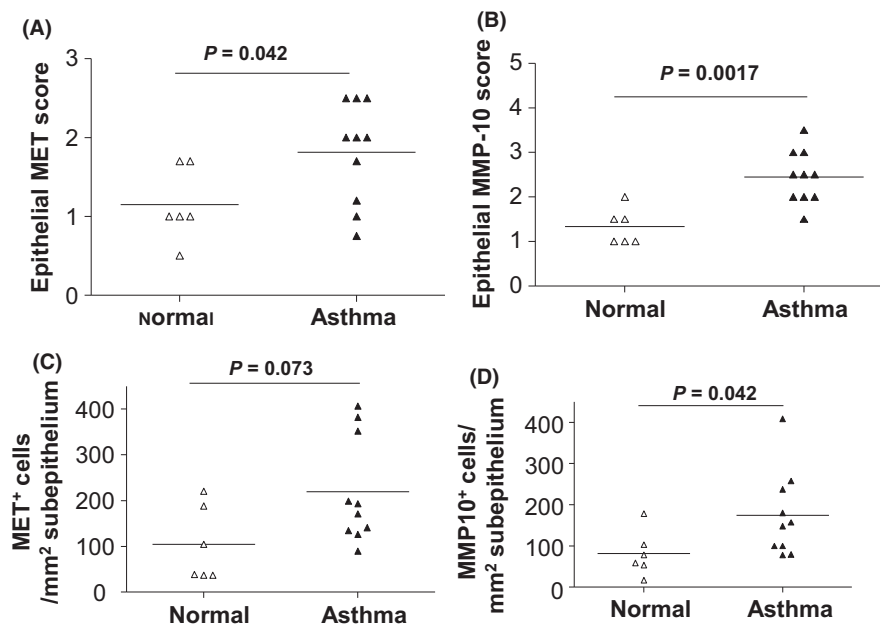


FIGURE 4 Scores of epithelial staining intensity for MET (A) and MMP10 (B) in bronchial biopsies of normal nonasthmatic (Δ) and asthmatic subjects ($\blacktriangle$). Counts for subepithelial MET⁺ (C) and MMP10⁺ (D) cells in bronchial biopsies of normal and asthmatic subjects. Data are expressed as the number of positive cells per mm² of subepithelium. Individual scores and counts are shown with horizontal bars showing median values

In the analysis of the regulatory factors for MMP-10 and MET, two transcription factors, ETS-1 and SOX (SRY-related HMG-box) family proteins, were identified. SOX proteins are involved in processes such as apoptosis, tumorigenesis, and remodeling³⁸ and, as such, may be implicated in airway remodeling in severe eosinophilic asthma. SOX4, 7, and 15 are part of the human large airway basal cell gene signature.³⁹ SOX4 has also been implicated in lung epithelial cell-mediated host defense against bacteria by regulating the expression of antimicrobial factors such as lysozyme.⁴⁰ Furthermore, SOX7 is involved in basal keratinocyte function and in epidermal-dermal junction plasticity and remodeling in the skin.⁴¹ Our data highlighted an inverse correlation between ETS1 and NKX3A, and MMP10 expression. ETS1 is associated with upregulation of profibrotic genes controlled by the TGF β signaling pathway in models of lung fibrosis⁴² and mediates TNF- α -induced MMP9 and tenascin expression in primary human bronchial fibroblasts, thus potentially playing a key role in airway remodeling.⁴³ ETS1 may also drive VEGF-induced MMP10 expression and endothelial cell migration and tube formation in vitro,⁴⁴ suggesting that the ETS1/MMP10 axis may also be involved in the angiogenesis of severe asthma.⁴⁵ ETS1 may also have a role in the regulation of MET expression.⁴⁶ Finally, ETS1 may provide a link between eosinophilia and remodeling as ETS1 can co-operate with GATA3 to induce IL-5 expression in human Jurkat T cells⁴⁷ and regulates GM-CSF production from human mast cells.⁴⁸ NKX3A is a homeobox-containing transcription factor that acts as a negative regulator of epithelial cell growth in prostate tissue and is a major driver of prostate cancer.⁴⁹ Future studies should examine the functional effects of ETS1 and SOX4, 7, and 15 on MMP10 and MET expression in primary basal airway epithelial cells and in animal models of severe eosinophilic asthma.

One of the strengths of the study has been the ability to relate mucosal eosinophils with its transcriptomic profile in order to attempt to define the role of the eosinophil in the remodeling process. However, there are some potential limitations of the data. First, there were relatively lower levels of mucosal eosinophil counts found in the asthmatic subjects compared to other similar cohorts,^{50,51} but this could be explained by the high-dose inhaled corticotherapy with a high proportion on daily oral corticosteroid therapy. Second, there was a relatively high level of eosinophil counts in the control nonasthmatic subjects that may be related to their level of atopic background; however, we did not use the comparison of asthmatic subjects to the control subjects because the hierarchical analysis did not yield significant differentiation of genes. Finally, we have not been able to examine the potential contribution of other inflammatory cells apart from eosinophils that could contribute to the complex process of airway wall remodeling. However, using GSVA, we found that there was little difference in expression levels of activation gene signatures for neutrophils, macrophages, and CD4⁺ T cells between the mucosal eosinophil-high and eosinophil-low patients (data not shown). A final limitation of our study is that we have not been able to validate our findings in an independent cohort, including a functional validation of MMP10 and MET in severe asthma.

In summary, asthma characterized by submucosal airway eosinophilic inflammation is associated with increased subbasement membrane thickness with MMP-10 and MET identified as most differentially expressed genes, and their regulatory factors comprise ETS-1 and SOX. These findings indicate the potential involvement of these factors underlying the submucosal eosinophilic inflammation and subepithelial fibrosis observed in asthma that warrant further investigation.

ACKNOWLEDGMENTS

The authors would like to thank all the U-BIOPRED team. Please see Appendix S1 for the individual list and groups in U-BIOPRED.

CONFLICTS OF INTEREST

Dr. Djukanovic reports personal fees from TEVA, grants and personal fees from Novartis, and personal fees and other support from Synairgen, outside the submitted work. Dr. Howarth reports part-time employment by GSK as Global Medical Expert. Dr. Krug, Dr. Kuo, Dr. Wilson, Dr. Guo, Dr. Pandis, Dr. Pavlidis, Dr. Gibeon, Dr. Hoda, Dr. Fowler, Dr. Zhu, Dr. Corfield, and Dr. Sousa have nothing to disclose. Dr. Chung reports personal fees from advisory board membership, grants for research, and personal fees from payments for lectures, outside the submitted work. Dr. F. Baribaud is a current employee and shareholder of Janssen R&D. Dr. Dahlén reports personal fees from advisory board membership and personal fees from payments for lectures, outside the submitted work. Dr. Auffray reports grants from Innovative Medicine Initiative and grants from Innovative Medicine Initiative, during the conduct of the study. Dr. Loza reports other from Janssen R&D, outside the submitted work. Dr. Shaw reports personal fees from advisory board meetings, outside the submitted work. Dr. Sterk reports grants from Innovative Medicines Initiative (IMI), during the conduct of the study. Dr. Chanez had consultancy services for Boehringer Ingelheim, Johnson & Johnson, GlaxoSmithKline, Merck Sharp & Dohme, AstraZeneca, Novartis, Teva, Chiesi, Sanofi, and SNCF; served on advisory boards for Almirall, Boehringer Ingelheim, Johnson & Johnson, GlaxoSmithKline, AstraZeneca, Novartis, Teva, Chiesi, and Sanofi; received lecture fees from Boehringer Ingelheim, Centocor, GlaxoSmithKline, AstraZeneca, Novartis, Teva, Chiesi, Boston Scientific, and ALK; and received industry-sponsored grants from Roche, Boston Scientific, Boehringer Ingelheim, Centocor, GlaxoSmithKline, AstraZeneca, ALK, Novartis, Teva, and Chiesi. Dr. A. Rowe is a current employee and shareholder of Janssen R&D. Dr. Adcock reports personal fees from advisory board membership, grants from grants, and personal fees from payments for lectures, outside the submitted work. Dr. Sandström reports personal fees from advisory board membership and personal fees from payments for lectures, outside the submitted work. Dr. Fleming reports personal fees from advisory board membership, grants for research, and personal fees from payments for lectures, outside the submitted work.

AUTHOR CONTRIBUTIONS

CSK, SP, ML, and FB performed the analysis. KFC, IMA, AR, KS, CA, and YG designed the analytical approaches taken and analyzed the results. DG, UH, PH, DS, SF, BD, PC, NK, TS, LF, PHH, MC, LF, SJW, and BD participated in the clinical characterization of the patients. IP, AS, and JC were part of the data curation team. IMA, RD, PJS, and KFC conceived of and designed the study. KFC, IMA, and CSK drafted the manuscript.

DATA REPOSITORY

The transcriptomic data have been deposited in the Gene Expression Omnibus database, <http://www.ncbi.nlm.nih.gov/geo> (accession no. GSE76225 for gene expression data of bronchial biopsies).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Kuo C-HS, Pavlidis S, Zhu J, et al.; On behalf of the U-BIOPRED Project Team. Contribution of airway eosinophils in airway wall remodeling in asthma: Role of MMP-10 and MET. *Allergy.* 2019;74:1102-1112. <https://doi.org/10.1111/all.13727>

**Contribution of airway eosinophils in airway wall remodeling in asthma: role of
MMP-10 and *MET***

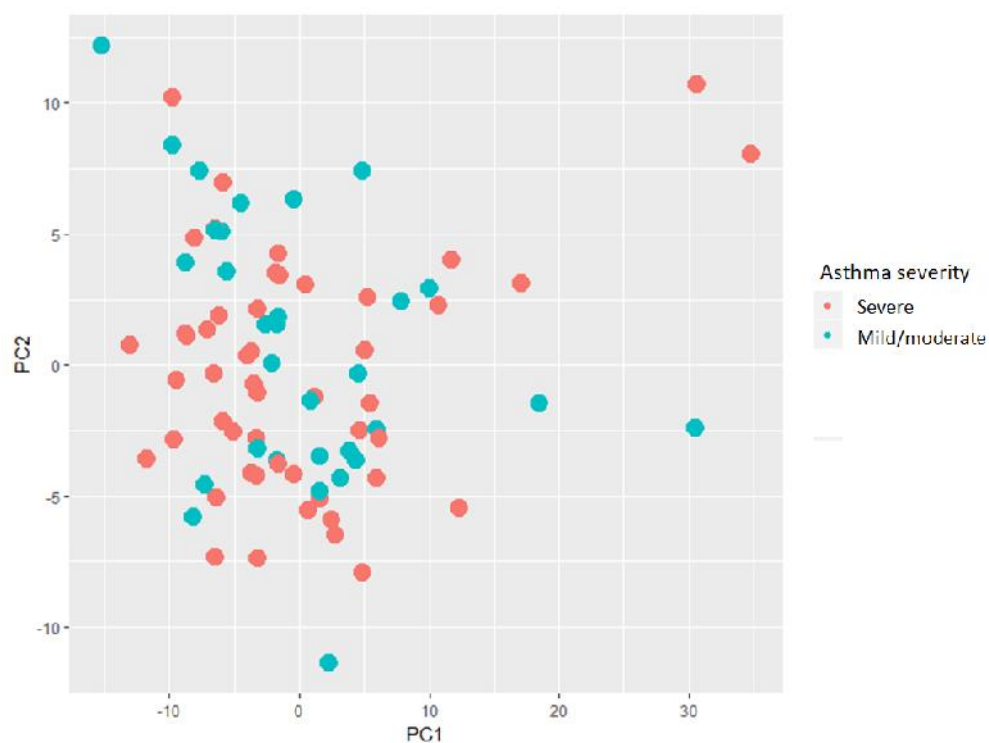
Chih-Hsi Scott Kuo, Stelios Pavlidis, Jie Zhu, Matthew Loza, Fred Baribaud, Anthony Rowe, Ioannis Pandis, David Gibeon, Uruj Hoda, Ana Sousa, Susan J. Wilson, Peter Howarth, Dominick Shaw, Stephen Fowler, Barbro Dahlen, Pascal Chanez, Norbert Krug, Thomas Sandstrom, Louise Fleming, Julie Corfield, Charles Auffray, Ratko Djukanovic, Peter J. Sterk, Yike Guo, Ian M. Adcock & Kian Fan Chung on behalf of the U-BIOPRED Project Team[#]

Supplementary data:

Figures S1, S2, S3, S4

Tables S1, S2, S3, S4, S5, S6

Figure S1



Legend:

Fig S1: Principle component analysis using the 251 differentially expressed genes characterizing the asthmatic cohort. Data points are colored by the severity of asthma.

Figure S2

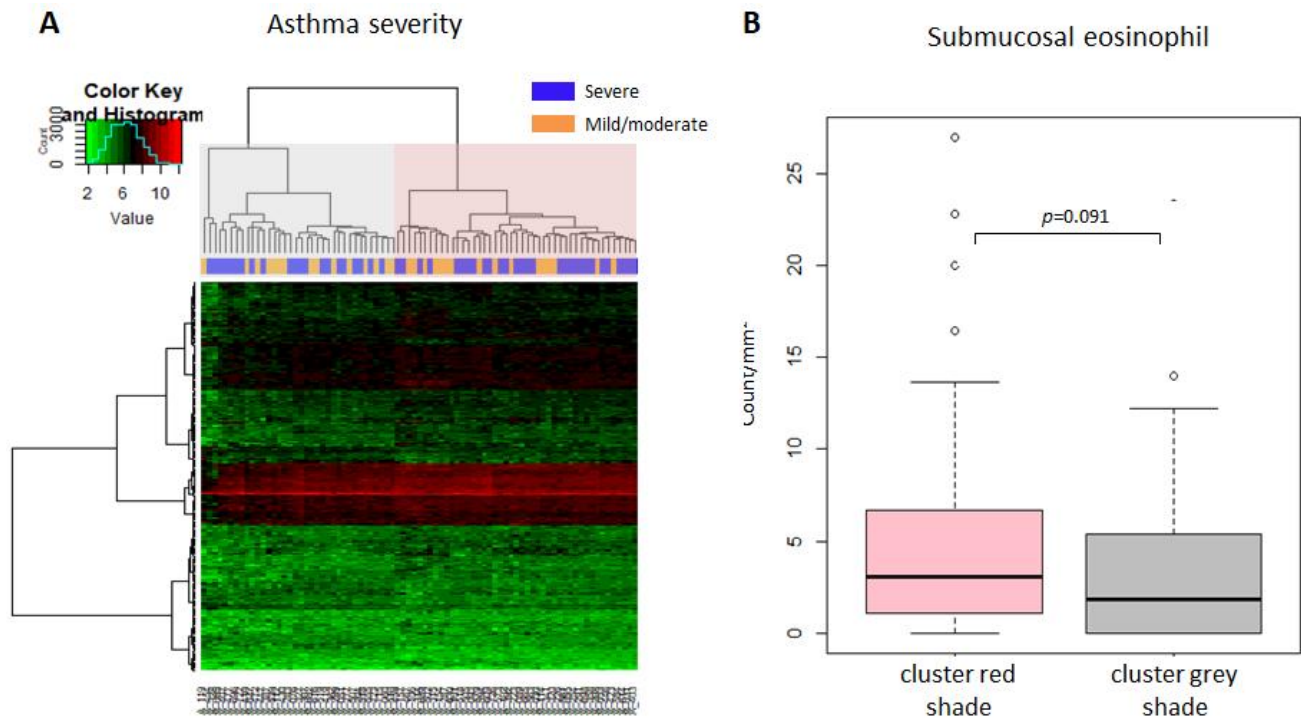


Fig S2. (A) Subgroup pattern of the 2 clusters identified from the 251 differentially-expressed genes (grey and red shade). No enrichment of the severe (blue) and mild/moderate (orange) asthmatic subjects was noted in either cluster (Chi-square test $p=0.740$). (B) Comparison of the submucosal eosinophil profile of the 2 clusters.

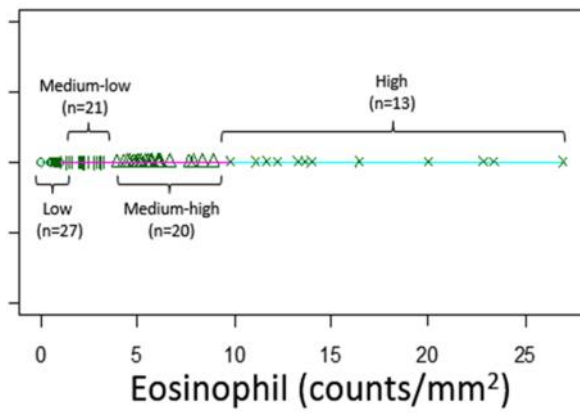
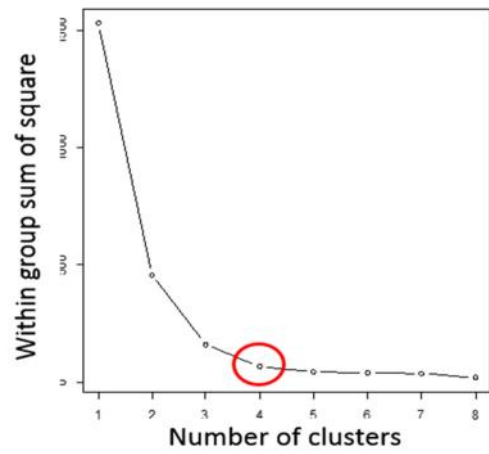
A**B****Fig S3**

Figure S3: (A) Four clusters were identified by K-mean clustering based on the count of submucosal eosinophils, where low and medium-low were merged as LE group, and medium-high and high as HE group. (B) Determination of the cluster numbers, as judged by the within-group sum of squares, where the cluster number beyond the bending point yielded minimal reduction of this value.

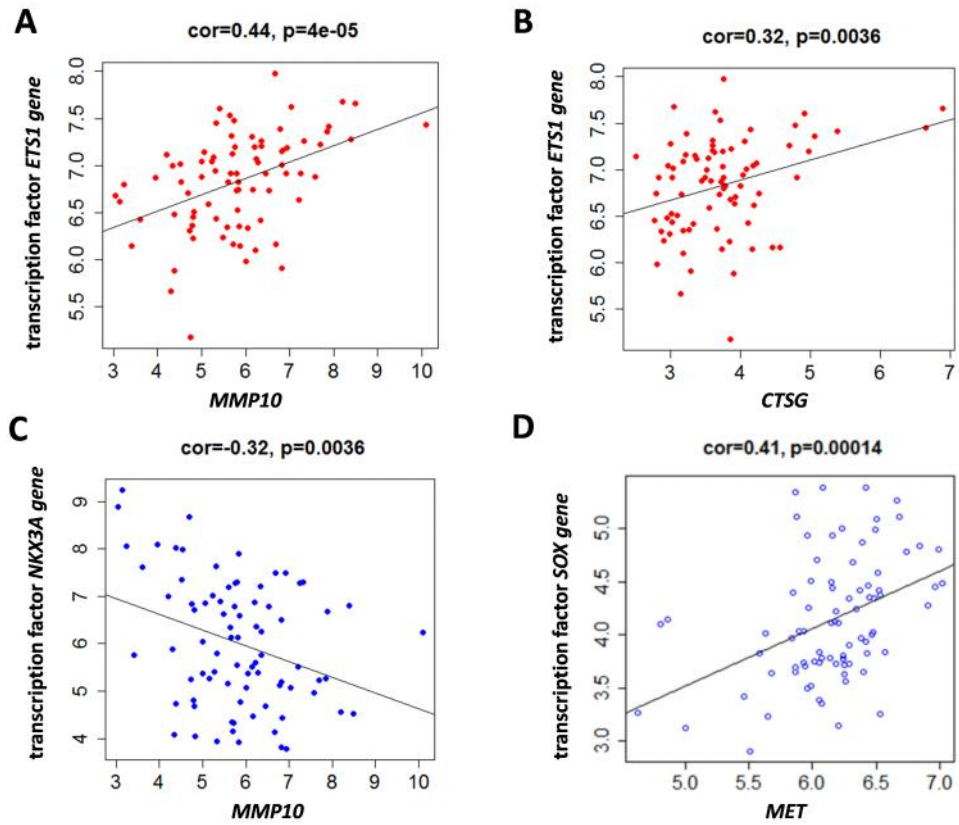


Fig S4

Figure S4: Relationship between ECM genes and genes of transcription factors *ETS1*, *NKX3A* and *SOX*. Positive correlation between *ETS1* and *MMP10* (Pearson's $r=0.44$, $p=4.0 \times 10^{-5}$) (A) and *ETS1* and *CTSG* (Pearson's $r=0.32$, $p=0.0036$) (B). Negative correlation between *NKX3A* and *MMP10* (Pearson's $r=-0.32$, $p=0.0036$) (C) and positive correlation between *SOX* and *MET* (Pearson's $r=0.41$, $p=1.4 \times 10^{-4}$) (D).

Table S1. Demographic and clinical characteristics of 81 asthmatics and 41 healthy volunteers

Variables[†]	Asthmatics N=81	Healthy volunteers N=41	p-value
Age (SD)	47.3±13.6	40.0±13.7	0.006
Female	41 (50.6)	13 (31.7)	0.073
BMI	29.0±5.7	25.2±3.5	<0.001
Nasal polyp	34 (32.7)	3 (7.3)	0.038
Allergic rhinitis	42 (40.4)	1 (2.4)	<0.001
Eczema	33 (31.7)	1 (2.4)	<0.001
Severe asthma	50 (61.7)	NA	NA
Oral corticosteroid use	22 (27.2)	NA	NA
Atopy	73 (70.2)	14 (34.1)	<0.001
Exacerbations (per year)	2.0 (1.0-3.8)	NA	NA
FEV ₁ (% predicted)	74.2 (60.6-93.3)	103.7(97.4-110.2)	<0.001
Total serum IgE (IU/ml)	104.5 (42.0-312.2)	19.9 (7.0-58.6)	<0.001
Blood leukocyte (10 ³ /μl)	7.50 (5.60-8.50)	4.90 (4.30-6.20)	<0.001
Blood eosinophil (10 ³ /μl)	0.20 (0.10-0.30)	0.10 (0.09-0.20)	<0.001
Blood neutrophil (10 ³ /μl)	4.40 (3.20-5.90)	2.70 (2.20-3.70)	<0.001
Sputum eosinophil (%)	1.1 (0.1-8.1)	0 (0-0.2)	<0.001
Sputum neutrophil (%)	52.7 (37.3-70.8)	25.3 (16.4-44.5)	<0.001
FeNO (ppb)	23.5 (16.9-47.3)	18.3 (14.6-29.0)	0.019
Serum periostin (ng/ml)	43.9 (39.2-51.6)	50.9 (45.4-58.4)	0.007
Serum CRP (mg/l)	2.0 (1.0-5.0)	1.0 (1.0-2.0)	0.004

[†]Data presented as N (%) and mean (SD) or median (IQR). BMI: Body mass index; CRP: C-reactive protein; FeNO: Fractional exhaled nitric oxide; FEV₁: Forced expiratory volume in 1 second; NA: Not applicable.

Table S2. Top 10 over-represented pathways from public ontology databases

Database ID	Name	<i>p</i> -value [#]
GO:0030198	Extracellular matrix organization	2.2x10 ⁻⁵
GO:0001578	Microtubule bundle formation	4.2x10 ⁻⁴
GO:0002696	Positive regulation of leukocyte activation	2.3 x10 ⁻³
GO:0034341	Response to interferon-gamma	2.7x10 ⁻³
GO:0042517	Immunoglobulin complex, circulating	2.8x10 ⁻³
GO:0048020	CCR chemokine receptor binding	1.4x10 ⁻²
GO:0002252	Immune effector process	3.5x10 ⁻²
KEGG:05146	Amoebiasis	3.6x10 ⁻³
KEGG:05310	Asthma	1.0x10 ⁻²
REAC:1474244	Extracellular matrix organization	1.1x10 ⁻⁴

Table S3: Breakdown of genes of six functional annotations used as signatures for gene set variation analysis (GSVA)

Name of signature	Up/Down regulate genes
Extracellular matrix organization	Up: <i>MMP10, MET, COL3A1, SPP1, POSTN, CTSG, VCAM1, TNC, TPSAB1</i> Down: <i>PCOLCE2, MFAP5</i>
Mast cell activation	Up: <i>HDC, IL33, MS4A2, CPA3</i>
CC chemokine	Up: <i>CCL26, CCL11, HLA-DQB1, CCL19, UBD, CCL2</i>
Immunoglobulin complex	Up: <i>IGHG3, IGHA1, IGH, IGJ, IGKC</i>
Serine proteinase inhibitors	Up: <i>SERPINB2, SERPINB10, SERPINB13</i>
Microtubule formation	Up: <i>DNAI2, SPEF2, LRRC6, DNAH7, AK7</i>

Table S4. Clinical features and submucosal cell profiles of the HE versus LE group

Variables	HE (n=33)	LE (n=48)	Missing data, n (%)	p-value
Age (years)	46.9±13.5	47.6±13.8	-	0.841
Female	15 (45.5)	26 (54.2)	-	0.586
BMI	27.7±4.7	29.9±6.3	-	0.074
Smoking	4 (12.1)	10 (20.8)	-	0.472
Nasal polyp	6 (18.2)	14 (29.2)	-	0.387
Allergic rhinitis	19 (57.6)	21 (43.8)	-	0.319
Eczema	12 (36.4)	20 (41.7)	-	0.804
Severe asthma	20 (60.6)	30 (62.5)	-	1.000
Oral corticosteroid use	10 (30.3)	12 (25.0)	-	0.785
Atopy	21 (63.6)	39 (81.3)	-	0.129
Exacerbations (per year)	3.0 (2.0-4.0)	2.0 (1.0-3.0)	39 (48.1)	0.123
FEV ₁ (% predicted)	73.5 (57.2-90.4)	77.7 (61.1-94.3)	-	0.331
Total serum IgE (IU/ml)	135.0 (57.0-338.2)	76.4 (38.3-232.0)	1 (1.2)	0.190
Blood eosinophil (10 ³ /μl)	0.26 (0.20-0.40)	0.15 (0.10-0.28)	-	0.005
Sputum eosinophil (%)	1.02 (0.22-6.07)	1.21 (0-9.04)	43 (53.1)	0.801
FeNO (ppb)	32.5 (20.0-57.0)	21.0 (14.5-42.3)	5 (6.2)	0.129
Serum periostin (ng/ml)	44.0 (40.4-52.6)	43.9 (38.9-50.9)	11 (13.6)	0.533
Serum CRP (mg/l)	1.0 (1.0-3.5)	4.2 (1.0-5.2)	4 (4.9)	0.043
<i>Submucosal cell counts</i>				
Eosinophil (mm ⁻³)	7.7 (5.5-12.2)	0.8 (0-2.1)	-	2.2x10 ⁻⁹
Neutrophil (mm ⁻³)	14.5 (7.4-20.6)	11.3 (6.8-15.3)	-	0.130
Macrophage (mm ⁻³)	4.0 (2.2-7.9)	2.0 (0.7-3.3)	-	4.7x10 ⁻⁴
CD4 T cell (mm ⁻³)	12.8 (8.0-23.9)	6.6 (3.3-11.8)	-	0.003
CD8 T cell (mm ⁻³)	21.0 (13.6-34.7)	12.7 (4.4-22.5)	-	0.016
Mast cell (mm ⁻³)	22.8 (17.1-35.5)	17.0 (8.6-26.7)	-	0.052
ASM fraction (%)	0.32 (0.25-0.38)	0.33 (0.22-0.44)	-	0.840
Thickness (μm)	9.2 (8.0-10.5)	8.2 (7.0-9.7)	13 (16.0)	0.012

Data presented as N (%) and mean (SD) or median (IQR). ASM: Airway smooth muscle; BMI: Body mass index; CRP: C-reactive protein; EOS: eosinophil; FeNO: Fractional exhaled nitric oxide; FEV₁: Forced expiratory volume in 1 second; IgE: Immunoglobulin E.

Table S5: Top over-represented pathways of the HE group in severe and mild/moderate asthma

Pathway Name	Pathway ID	P-value
<i>Severe asthma</i>		
extracellular matrix organization	REAC:1474244	0.0027
axonemal dynein complex assembly	GO:0070286	0.00652
microtubule bundle formation	GO:0001578	0.0145
extracellular matrix organization	GO:0030198	0.022
extracellular structure organization	GO:0043062	0.0225
proteinaceous extracellular matrix	GO:0005578	0.0445
outer dynein arm assembly	GO:0036158	0.0493
<i>Mild/moderate asthma</i>		
extracellular matrix organization	REAC:1474244	9.21E-08
collagen biosynthesis and modifying enzymes	REAC:1650814	1.28E-05
cell migration	GO:0016477	2.50E-05
protein digestion and absorption	KEGG:04974	4.81E-05
proteinaceous extracellular matrix	GO:0005578	4.82E-05
signaling by interleukins	REAC:449147	4.99E-05
assembly of collagen fibrils and other multimeric structures	REAC:2022090	5.15E-05
interleukin-7 signaling	REAC:1266695	8.99E-05

Table S6: Transcription factor binding site analysis of 8 upregulated ECM genes

Transcription factor	Number of gene	p-value	Enrichment factor	Gene (genomic coordinate of binding site relative to TSS)
M01032[HNF4]	5	0.035	2.105	SPP1(-416), MET(-970,-674), VCAM1(-387), POSTN(-301,-5), COL3A1(113)
M00478[Cdc5]	2	0.035	3.283	POSTN(-569,-126), MMP10(-112)
M00451[NKX3A]	4	0.023	4.951	CTSG(-437), SPP1(-376), VCAM1(-741), MMP10(-251)
M00206[HNF-1]	2	0.033	3.309	VCAM1(-862,-525), POSTN(-402)
M00339[c-Ets-1]	3	0.022	3.231	SPP1(-153,-127), CTSG(-337), MMP10(-190)
M00482[PITX2]	2	0.002	4.184	CTSG(-924,-359,-171), MMP10(-178)
M00145[Brn-2]	2	0.034	3.368	VCAM1(-965), MMP10(-787,-381)
M00462[GATA-6]	4	0.003	7.426	CTSG(-425), MET(169), VCAM1(-280), COL3A1(-559)
M00042[Sox-5]	3	0.008	4.607	SPP1(-399), MET(-681), POSTN(-640,-285)
M00992[FOXP3]	3	0.014	6.37	VCAM1(-735), COL3A1(-163), MMP10(-916)
M00216[TATA]	4	0.002	8.427	CTSG(-977), SPP1(-905), VCAM1(-225), MMP10(-33)
M00148[SRY]	4	0.006	2.089	SPP1(-310,-135,-125), VCAM1(-677), POSTN(-405,-350,-53), MMP10(-440)
M01000[AIRE]	4	0.001	5.91	CTSG(-848), VCAM1(-824,-712), POSTN(-298), MMP10(-359)
M00436[IPF1]	2	0.007	3.224	CTSG(-369), SPP1(-921,-897,-515)
M00203[GATA-X]	3	0.012	6.889	CTSG(-423), COL3A1(-557), TNC(-375)
M01081[Zec]	2	0.049	2.696	SPP1(-355), TNC(-961,84)
M00490[Bach2]	3	0.047	4.047	VCAM1(-515), TNC(-781), MMP10(-69)
M00138[Oct-1]	4	0.005	3.169	SPP1(-555,-294), VCAM1(-741,-569), POSTN(-108), COL3A1(-798,-780)
M00081[Evi-1]	3	0.044	2.864	SPP1(-128), POSTN(-538), COL3A1(-795,-165)
M00499[STAT5A]	2	0.011	2.724	SPP1(-981), COL3A1(-432,-210,-60)
M00941[MEF-2]	2	0.031	3.478	POSTN(-124,-70), MMP10(-348)
M00619[Alx-4]	2	0.009	14.575	POSTN(-286), TNC(-820)
M00284[TCF11:MafG]	3	0.028	4.994	VCAM1(-519), COL3A1(-899), MMP10(-927)
M00456[FAC1]	4	0.023	4.578	CTSG(-244), SPP1(-137), POSTN(-58), COL3A1(-100)

M00987[FOXP1]	5	9.54E-04	2.9	SPP1(-855,-470,-146,-138), VCAM1(-733,-561), POSTN(-639,-63), COL3A1(-372,-68), MMP10(-479)
M01014[SOX]	4	0.002	7.531	MET(26), POSTN(-952), COL3A1(136), TNC(-535)
M00424[NKX6-1]	3	0.018	3.936	VCAM1(-822), POSTN(-765), MMP10(-658,-113)

TSS: transcription start site

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Supplementary file of Acknowledgements

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The U-BIOPRED consortium wishes to acknowledge the help and expertise of the following individuals and groups without whom, the study would not have been possible:

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